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AGONISTIC BEHAVIOR BETWEEN RECENTLY COLLECTED AND LABORATORY CULTURED *RETICULITERMES* SPP. (ISOPTERA: RHINOTERMITIDAE) FROM NORTHERN CALIFORNIA

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Abstract.—Mixing workers from different foraging groups of the same colony of *Reticulitermes* spp. from northern California never resulted in agonistic behavior and seldom (8%) resulted in mortality after 24 h. When colonymates are reunited, behaviors such as head and body tapping and antennation of one another are observed. Intermingling workers from different colonies of the same cuticular hydrocarbon phenotype normally does not result in immediate aggression, but mortality is usually high (83%) after 24 h. Attempts to mix workers from colonies with different cuticular hydrocarbon phenotypes result in immediate aggressive behavior 88% of the time and high mortality (≥ 50%) 100% of the time after 24 h. Mixing workers from cultures of *Reticulitermes* spp. maintained in the laboratory for > 18 months with workers recently collected from the same colony in the field resulted in neither obvious agonistic behavior nor significant mortality (< 5%) after 24 h. Commingling workers from laboratory cultures with workers recently collected from different field colonies of the same cuticular hydrocarbon phenotype usually resulted in high mortality (42 of 54 bioassays with > 50% mortality). Interactions between workers from laboratory cultures with workers recently collected from field colonies of a different cuticular hydrocarbon phenotype always resulted in high mortality. These results suggest that termites separated from their colony and maintained in the laboratory for > 18 months continue to recognize colonymates from the field, and vice versa. Maintenance of laboratory cultures of *Reticulitermes* colonies can be a valuable tool to test the efficacy of baits by determining if a colony has been successfully eliminated, has avoided the baits and subsequently returned to the monitors, or has been replaced by a completely different colony.

Key Words.—Insecta, *Reticulitermes,* aggression, baiting, fighting, monitoring, subterranean termites.

Intraspecific and interspecific aggression between or among termites has been investigated as a means of deducing relationships among colonies of termites (Thorne & Haverty 1991, Shelton & Grace 1996). Intraspecific and interspecific agonism is the norm, however, passive intraspecific encounters have been recorded for *Reticulitermes santonensis* Feytaud (Clément 1986), *R. flavipes* (Kollar) (Grace 1996, Polizzi & Forschler 1998), *R. virginicus* (Banks) (Polizzi & Forschler 1998), *Coptotermes formosanus* Shiraki (Su & Haverty 1991, Shelton 1996), and for three species of *Zootermopsis, Z. nevadensis* (Hagen), *Z. angusticollis* (Hagen), and *Z. laticeps* (Banks) (Thorne & Haverty 1989). Agonism has been used to infer colony affiliation of foraging groups or satellite groups of the subterranean termites *Heterotermes aureus* (Snyder) (Binder 1988, Jones 1990), *Reticulitermes* spp. (Haverty et al. 1999a), *Reticulitermes* (*l.*) *banyulensis* Clément (Clément 1980), and *R.* (*l.*) *grassei* Clément and *R.* (*l.*) *lucifugus* Rossi (Clément 1986).

We studied seasonal foraging and feeding behavior and the size and dispersion of colonies of *Reticulitermes* spp. in northern California as background information to assist in the evaluation of baits for control of these subterranean termites (Haverty et al. 1999b, 2000). The same color stain was used to mark termites in monitoring stations throughout each site. This led to difficulties determining termite associations in the numerous monitoring stations at our research sites (Lewis et al. 1998). Haverty et al. (1999a) proposed an approach that would first characterize the cuticular hydrocarbons of termites in each foraging group collected from each monitoring station on each observation date. If the hydrocarbon phenotypes are different, then the termites are from different colonies. When the hydrocarbon phenotypes are the same, then agonistic bioassays would be started. Termites that are aggressive toward one another are assumed to be from different colonies. Finally, if the termites are from the same cuticular hydrocarbon phenotype and do not react aggressively, then the next step would be to initiate a mark-release-recapture (MRR) to document if there is a connection between the groups. This integrated approach would reduce reliance on use of dyes alone to assess the relationship of foragers from different monitoring stations (Haverty et al. 1999a). However, it should be pointed out that not all distinct colonies of subterranean termites react aggressively towards one another and that certain pairings of distinct colonies sometimes result in high levels of mortality (> 50%) and sometimes they do not (Haverty et al. 1999a, Su & Haverty 1991, Thorne & Haverty 1991, Shelton 1996).

Problems can occur when evaluating baits for control of subterranean termites. If termites appear in monitoring devices within the territory or foraging area occupied by the colony that was presumably eliminated or suppressed, are they members of the original colony, or are they new immigrants into the area? The same protocol proposed by Haverty et al. (1999a) can be modified to address this question. The cuticular hydrocarbon phenotype of the colony treated with bait must be documented before treatment. If the termites appearing in monitoring devices following the bait treatment are of a different cuticular hydrocarbon phenotype, then it is clear that a different colony has become established in the territory of the baited colony. If the cuticular hydrocarbon phenotype of the previous and recent termites are the same, then an agonistic bioassay could be used to determine whether the termites are from the original colony. This would require, however, maintaining a laboratory culture of the original colony.

We report here the results of laboratory bioassays to assess the aggressive behavior of recently collected termites toward termites kept in culture in the laboratory for more than 18 months. We suggest this protocol as a means to determine the possible origin of termites appearing in the territory of a colony presumably eliminated by a bait treatment.

Materials and Methods

Collections of *Reticulitermes* were made from one wildland location and two residential locations in northern California (Haverty et al. 1999a, b, 2000). The wildland site was the Institute of Forest Genetics (IFG) near Placerville, El Dorado County, California. This is ~4 ha and composed of a 70-year-old plantation of mixed *Pinus* spps. The residential sites were in Marin County: one each in Novato and Larkspur. The Novato site (St. Francis of Assisi Church) consists of

a single-family dwelling (the church rectory), the church, and extensive gardens, walks, and large trees on a 1-ha lot (Lewis et al. 1998). The Larkspur site is a single-family, 62-year-old residence.

We installed monitoring stations from which we collected foragers on a monthly basis from 1993 through 1996 (Lewis et al. 1998). Sixty-eight stations were installed at IFG, 34 at Novato, and 12 at Larkspur. Separate laboratory cultures were established from foraging termites collected from each monitoring station through December 1996. Cultures were augmented each month with foragers from the same monitoring station under the assumption that a given monitoring station was consistently occupied by one colony over the duration of the study. Thus, each individual colony might have multiple cultures because some colonies occupied more than one monitoring station (Haverty et al. 2000). Cultures were maintained in the laboratory for up to 36 months in containers provided with sand/vermiculite/water (1:1:0.8 vol.) (Haverty 1979). Cultures were supplied wood from old monitoring station bundles (Lewis et al. 1998) and remoistened as needed.

Termites were not collected between 1 Jan 1997, and 14 Jun 1998. However, in June 1998 collections were made again from the monitoring stations at IFG, Novato, and Larkspur. They were returned to the laboratory and placed in separate cultures from those collected prior to January 1997. Thus, we maintained old and new cultures in the laboratory for study.

To observe the behavior of colony members rejoined after > 18 months of separation, we paired two groups of 10 workers from the same monitoring station, each group from a different collection period: pre-1997 and post-1998. To ascertain the potential for aggression of these same cultures, we paired two groups of 10 workers, each group from a culture from a different monitoring station known to be used by different colonies; 75% of these pairings were from different collection periods, pre-1997 and post-1998, while 25% were from the same collection period, pre-1997.

The two groups of 10 workers were placed in plastic Petri dishes (5 cm diameter) with tight-fitting lids, provisioned with a 47-mm absorbent pad (Gelman Sciences, Ann Arbor, MI) moistened with one ml of distilled water (Haverty et al. 1999a). Cultures from a monitoring station were removed from the container and placed on a tray. As the workers walked away from the culture medium they were aspirated into a container until a group of 10 was collected. The groups of 10 were poured into the Petri dish. The second group was handled similarly. An equal number of replicates for all combinations was attempted, however, variations in the number of replications per treatment occurred due to insufficient number of workers available in laboratory cultures or monitoring stations.

Behavior was observed for ~two min upon combining the two groups to record immediate aggression (Haverty et al. 1999a). Surviving termites were counted after 24 h. These groups of 20 termites were kept in the Petri dishes in the laboratory under ambient conditions for 24 h. Mortality was considered high if 10 or fewer live termites remained, low if 17 or more remained and equivocal if 11 to 16 termites remained after 24 h (Haverty et al. 1999a).

RESULTS

Cultures from the Same Monitoring Station, Different Collection Period.—Behavioral observations yielded the same results in all 54 pairings. No aggressive

behavior, such as biting or lunging, was observed in any of these pairings. Seventeen of the 215 pairings of cultures from monitoring stations from IFG had 19 termites surviving; the remaining 92.1% of the paired groups of 10 had all 20 termites surviving after 24 h (Table 1). Similar results were observed with the termites from the residential sites. Only two of the 80 paired groups had 19 termites surviving after 24 h; all of the other paired groups had 20 survivors after 24 h.

Cultures from Different Colonies, Same or Different Collection Period.—Agonistic responses resulting from different combinations (collection dates, colony affiliations, or hydrocarbon phenotype) were observed with the assumption that none of the pairings were from the same colony. These tests were conducted to confirm the robustness of termites in culture or from the field and to provide a positive control. When paired with known antagonist termite colonies, they would indeed fight. High mortality (≥ 50%) resulted 92% of the time when groups of 10 workers from different colonies were paired (Table 2).

Mixing cultures from the same phenotype resulted in high mortality (≥ 50%) 83% of the time. However, certain pairings, such as Wc26 vs. Wg36, Wb33 vs. Wg36, Wg36 vs. Wh54, Wg46 vs. Wt46, and Wg46 vs. Wt51, resulted in equivocal mortality; sometimes it was high and sometimes it was low (Table 2). These equivocal results in pairing different colonies of the same phenotype are expected (Haverty et al. 1999a). Combining cultures of different phenotypes, and thus obviously different colonies, resulted in aggressive behavior and high (≥ 50%) worker mortality 100% of the time (Table 2).

Discussion

The results of this study suggest that *Reticulitermes* from northern California can be maintained in culture in the laboratory for an extended period of time without losing the ability to distinguish colonymates from non-colony mates. These observations and conclusions do not differ from those made from pairings of groups of 10 workers from recent collections or contemporary laboratory cultures of the same populations sampled by Haverty et al. (1999a). We were careful to avoid cooling the termites when we transported them to the laboratory, as cooling has been reported to decrease aggression, even between different species (Dropkin 1946, Howick & Creffield 1980, Shelton & Grace 1997). The colonies we studied apparently were able to retain the factor(s) responsible for recognition, whether that was colony odor or the make-up of the cuticular hydrocarbons on the cuticle (Adams 1991, Haverty & Thorne 1989, Su & Haverty 1991, Thorne & Haverty 1991).

From these studies we conclude that laboratory cultures, maintained over time, retain their ability to recognize colonymates and non-colony mates. This characteristic could be important if agonistic behavior were used as a bioassay for determining if foraging groups of *Reticulitermes* from northern California belong to the same colony (Haverty et al. 1999a) as suggested for *Heterotermes aureus* (Snyder) (Jones 1990). Furthermore, laboratory cultures could be used to ascertain the affiliation of termites appearing in monitoring devices within the territory or foraging area occupied by a colony that was apparently eliminated or suppressed by baiting. Agonistic bioassays can be used to determine whether the colony was suppressed and subsequently resurged, or if newly collected termites are from a

Table 1. Number of survivors from pairings of two groups of 10 workers from the same monitoring station. One group is from collections taken prior to 1 Jan 1997 and the other group from collections taken after 1 Jul 1998.

Pairings[a]	No. survivors in each pairing[b]	Pairings[a]	No. survivors in each pairing[b]
IFG: Wc7	20, 20, 20, 20, 20, 20	IFG: Yr34	20, 20, 20, 20, 19, 20
IFG: Wc26	20, 20, 20, 20, 20, 20	IFG: Yv32	20, 20, 20, 20, 20, 20
IFG: Wb33	20, 20, 20, 20, 20, 20	IFG: Yv34	20, 20, 20, 20, 20, 20
IFG: Wg36	20, 20, 20, 20, 20, 20	IFG: Ze4	20, 20, 19, 20, 20, 20
IFG: Wt46	20, 20, 20, 20, 20, 20	IFG: Zp8	20, 20, 20, 20, 20, 20
IFG: Wt51	20, 20, 20, 19, 19, 20	IFG: Zn11	20, 20, 20, 20, 20, 20
IFG: Wh54	20, 19, 20, 19, 20, 20	IFG: Zm13	20, 20, 20, 20, 20, 19
IFG: Xt10	20, 19, 20, 20, 20, 20	IFG: Zp49	20, 20, 19, 20, 20, 20
IFG: Xo16	20, 20, 20, 20, 20, 20	IFG: Zs59	20, 20, 20, 20, 20, 20
IFG: Xi21	20, 20, 20, 19, 20, 20	IFG: Zq61	19, 20, 20, 20, 19, 20
IFG: Yt2	20, 20, 20, 20, 20, 20		
IFG: Yz16	19, 20, 19, 20, 20, 20	StF: 12	20, 20, 20, 20, 20, 19
IFG: YA16	20, 20, 20, 20, 20, 19	StF: 18	20, 20, 20, 20, 20
IFG: YD16	20, 20, 20, 20, 20, 20	StF: 21	20, 20, 20, 20, 20, 20
IFG: Yr19	20, 20, 20, 20, 20, 20	StF: 25	20, 20, 20, 20, 20
IFG: Ys19	20, 20, 20, 20, 20, 20	StF: 57	20, 20, 20, 20, 20, 20
IFG: Yt19	20, 20, 19, 20, 20, 20	StF: 87	20, 20, 20, 20, 20, 20
IFG: Yw20	20, 20, 20, 20, 20, 20	StF: 116	20, 20, 20, 20, 20, 20
IFG: YE20	20, 20, 20, 20, 20, 20	StF: 125	20, 20, 20, 20, 19, 20
IFG: Yr23	20, 20, 20, 20, 20	StF: 256	20, 20, 20, 20, 20, 20
IFG: Yw25	20, 20, 20, 20, 20, 20	StF: 314	20, 20, 20, 20, 20, 20
IFG: Yh27	20, 20, 20, 20, 20, 20	StF: 333	20, 20, 20, 20
IFG: Yk27	20, 20, 20, 20, 20, 20		
IFG: YI28	20, 20, 19, 20, 20, 20	L: 4	20, 20, 20, 20, 20, 20
IFG: Yh30	20, 20, 20, 20, 20, 20	L: 60	20, 20, 20, 20, 20, 20
IFG: Yk32	20, 20, 20, 20, 20, 20	L: 61	20, 20, 20, 20, 20, 20

[a] Monitoring stations from the Institute of Forest Genetics (IFG), St. Francis of Assissi Church in Novato (StF), or Larkspur (L).

[b] After 24 h, the number of workers alive in the bioassay arena for each pairing. For example, in IFG: Wc7 there were 6 pairings, each with 20 workers surviving. Each pairing was with 10 workers collected prior to January 1, 1997 and 10 workers collected from the same monitoring station after July 1, 1998.

Table 2. Number of survivors from pairings of two groups of 10 workers from culture from different colonies from the same or different collection periods.

Pairings[a]	No. survivors in each pairing[b]	Pairings[a]	No. survivors in each pairing[b]
Same Phenotypes[c]		Different Phenotypes[c]	
IFG: Wc26 vs Wb33	1, 4, 1, 3, 1, 5	IFG: Wc26 vs Wb36*	3, 0, 0, 2, 1
IFG: Wc26 vs Wg36	11, 18, 3, 4	IFG: Wc26 vs Wb36	1, 0, 0, 1, 2, 0
IFG: Wb33 vs Wg36*	14, 0, 5, 0	IFG: Wb36 vs Wb33	3, 2, 2, 1, 5
IFG: Wg36 vs Wh54	10, 6, 10	IFG: Yt2 vs Yq23	2, 4, 4, 0, 5
IFG: Wg46 vs Wt46	16, 10, 20, 16, 17, 17	IFG: Yt2 vs Wb33	8, 8, 4, 8, 7
IFG: Wg46 vs Wt51	6, 0, 0, 2, 0, 1, 11, 1, 0	IFG: Yh27 vs Yt2	4, 4, 5, 2, 4
IFG: Wg46 vs Wh54*	8, 4, 0	IFG: Yh27 vs Yq23	6, 8
IFG: Wh54 vs Wt51	2, 4, 2, 2, 2, 0	IFG: Zn11 vs Wh54	0, 2, 0, 1, 2
IFG: Zn11 vs Yt2	3, 7, 6	IFG: Zn11 vs Yq23	2, 4, 4, 4, 4
StF: 66 vs 78	3, 5, 8, 3, 0, 7	StF: 66 vs 35	5, 4, 3, 2, 2
StF: 71 vs 78	1, 5		

[a] Monitoring stations from the Institute of Forest Genetics (IFG), St. Francis of Assisi Church in Novato (StF).

[b] The number of workers alive after 24 h in the bioassay arena for each pairing.

[c] Column one of each pair collected prior to 1 Jan 1997; column two of each pair collected after 1 June 1998 except those noted * were collected prior to 1 Jan 1997.

different colony that has now become established in the territory previously occupied by a different colony or colonies. To do this, the cuticular hydrocarbon phenotype of the original colonies treated with bait must be documented before treatment and a culture maintained. If the termites appearing in monitoring devices after completion of the bait treatment are of a different cuticular hydrocarbon phenotype, then it is clear that a different colony has become established in the territory of the previously baited colony. If the cuticular hydrocarbon phenotype of the previous and recent termites are the same, then an agonistic bioassay could be used to determine whether the termites are from the same colony.

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Literature Cited

Adams, E. S. 1991. Nest-mate recognition based on heritable odors in the termite *Microcerotermes arboreus*. Proc. Nat. Acad. Sci., 88: 2031–2034.

Binder, B. F. 1988. Intercolonial aggression in the subterranean termite *Heterotermes auxeus* (Isoptera: Rhinotermitidae). Psyche, 95: 123–137.

Clément, J.-L. 1980. Aggression intra- et interspecifique dans le genre *Reticulitermes* (Isoptera). Sequences comportementales de reconnaissance coloniale. Congres sur les Societes d'Insectes Senangue. Biol. Ecol. Mediterr., 7: 157–158.

Clément, J.-L. 1986. Open and closed societies in *Reticulitermes* termites (Isoptera: Rhinotermitidae): geographic and seasonal variations. Sociobiology 11: 311–323.

Dropkin, V. H. 1946. The use of mixed colonies of termites in the study of host-symbiont relations. J. Parasitol., 32: 247–251.

Grace, J. K. 1996. Absence of overt agonistic behavior in a northern population of *Reticulitermes flavipes* (Isoptera: Rhinotermitidae) from Canada. Sociobiology, 28: 103–110.

Haverty, M. I. 1979. Selection of tunneling substrates for laboratory studies with three subterranean termite species. Sociobiology, 4: 315–320.

Haverty, M. I. & B. L. Thorne. 1989. Agonistic behavior correlated with hydrocarbon phenotypes in dampwood termites, *Zootermopsis* (Isoptera: Termopsidae). J. Insect Behav., 2: 523–543.

Haverty, M. I., K. A. Copren, G. M. Getty & V. R. Lewis. 1999a. Agonistic behavior and cuticular hydrocarbon phenotypes of colonies of *Reticulitermes* (Isoptera: Rhinotermitidae) from northern California. Annals Entomol. Soc. Amer., 92: 269–277.

Haverty, M. I., G. M. Getty, K. A. Copren & V. R. Lewis. 1999b. Seasonal foraging and feeding behavior of *Reticulitermes* spp. (Isoptera: Rhinotermitidae) in a wildland and a residential location in northern California. Environ. Entomol., 28: 1077–1084.

Haverty, M. I., G. M. Getty, K. A. Copren & V. R. Lewis. 2000. Size and dispersion of colonies of *Reticulitermes* spp. (Isoptera: Rhinotermitidae) in a wildland and a residential location in northern California. Environ. Entomol., 29: 241–251.

Howick, C. D. & J. W. Creffield. 1980. Intraspecific antagonism in *Coptotermes acinaciformis* (Froggatt) (Isoptera: Rhinotermitidae). Bull. Ent., Res. 70: 17–23.

Jones, S. C. 1990. Delineation of *Heterotermes aureus* (Isoptera: Rhinotermitidae) foraging territories in a Sonoran Desert grassland. Environ. Entomol., 19: 1047–1054.

Lewis, V. R., M. I. Haverty, G. M. Getty, K. A. Copren & C. Fouche. 1998. Monitoring station for studying populations of *Reticulitermes* (Isoptera: Rhinotermitidae) in California. Pan-Pac. Entomol., 74: 121–133.

Polizzi, J. M. & B. T. Forschler. 1998. Intra- and interspecific agonism in *Reticulitermes flavipes*

(Kollar) and *R. virginicus* (Banks) and effects of arena and group size in laboratory assays. Insectes Soc., 45: 43–49.

Shelton, T. G. 1996. Factors affecting colony recognition in *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae). M.S. Thesis, University of Hawaii at Manoa, Hawaii.

Shelton, T. G. & J. K. Grace. 1996. Review of agonistic behaviors in the Isoptera. Sociobiology, 28: 155–176.

Shelton, T. G. & J. K. Grace. 1997. Impact of low temperature conditioning on intercolonial agonism in *Coptotermes formosanus* (Isoptera: Rhinotermitidae). Sociobiology, 30: 197–211.

Su, N.-Y. & M. I. Haverty. 1991. Agonistic behavior among colonies of the Formosan subterranean termite, *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae), from Florida and Hawaii: lack of correlation with cuticular hydrocarbon composition. J. Ins. Behav., 4: 115–128.

Thorne, B. L. & M. I. Haverty. 1991. A review of intracolony, intraspecific, and interspecific agonism in termites. Sociobiology, 19: 115–145.

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